medium that will maximize growth of the selected microorganism. In fact, the optimum pH and temperature that will support growth of the selected microorganism can be easily determined from a textbook of microbiology. Furthermore, not just any alternative oxidant is selected for use in the process. To the contrary, one having ordinary skill in the art would select an alternative oxidant source, based on the knowledge that the already-selected microorganism can utilize that alternative oxidant. For example, one having ordinary skill in the art would not select sulfur as the alternative oxidant, if she/he had already selected a microorganism that could only utilize sodium nitrate as the alternative oxidant source. In the just-described example, the skilled artisan would select sodium nitrate as the alternative oxidant source. Therefore, the selection of the appropriate alternative oxidant source, is based on the ability of the already-selected microorganism to utilize it. Again, one having ordinary skill in the art could determine the appropriate alternative oxidant source from a collegiate level textbook of microbiology.

The recitation "allowing the culture medium to incubate for a time sufficient to produce a desired quantity of biological product" is not indefinite. The incubation time that is employed is based on the amount of product that is desired to be formed during the process.

Applicants have not amended claim 1, as to amend claim 1 to recite a specific biological product, a specific microorganism and/or specific alternative oxidant would unduly limit the scope of the present invention. Applicants emphatically submit that claim 1 is clear to one having ordinary skill in the art. Applicants, therefore, respectfully request that the rejection of claims 1-34 and 71 be withdrawn.

It is alleged that claim 5 is an improper dependent claim for failing to further limit the subject matter of a previous claim. Applicants have canceled claim 5. Applicants, therefore, respectfully request that the objection to claim 5 be withdrawn.

It has been alleged that claim 6 is vague and indefinite in reciting the *Clostridium* and *Desulfovibrio* are facultative anaerobes. In response, Applicants have amended claim 6 by deleting *Clostridium* and *Desulfovibrio* from the claim. Claim 6 now reads as follows: "The process of claim 5, wherein the bacteria is selected from a genus selected from the group

consisting of *Pseudomonas, Paracoccus, Micrococcus, Klebsiella, Escherichia, Acidianus, Campylobacter, Wolinella,* and *Proteus."* The paragraph at page 11, lines 24-28 spanning page 12, lines 1-6 has been amended by deleting *Clostridium* and *Desulfovibrio* from the description of preferable facultative aerobes.

It has also been alleged that the Genus *Acidianus* is not recognized as a Genus of microorganism. Applicants have enclosed an except from *Brock Biology of Microorganisms*, which identifies the Genus Acidianus as a facultative aerobe. See Madigan, M.T., Brock Biology of Microorganisms, 8th Edition, Prentice Hall, Upper Saddle River, NJ, pp.758-759 (1997). Accordingly, the Genus *Acidianus* Applicants respectfully submit that the rejection of claim 6 is now moot.

It is alleged that claim 31 is confusing in the recitation of 50g/L as the cellular concentration for inoculation of the culture medium. Applicants respectfully traverse this rejection. It is very common for one having skill in the art to develop a calibration curve between cell concentration (as either g/L or number of cells/ml) in a culture and the optical density of the culture, by measuring the culture at a certain wavelength using a spectrophotometer. With a developed calibration curve, the optical density of a culture grown for inoculation purposes can be measured periodically to determine the cell concentration (in g/L) prior to inoculation. Inoculation can be made when the cell concentration reaches the desired level. A preferred range for cellular concentration for inoculation of the culture medium is from about 0.1 g/L to about 50 g/L of the desired microorganism. Applicants respectfully submit that the use of "about 0.1 g/L to about 50g/L" to describe a preferable range for the cell concentration that is used to inoculate the culture medium is clear to those having ordinary skill in the art. Applicants, therefore, respectfully request that the rejection of claim 31 be withdrawn.

35 U.S.C. §102(b) Rejection

Claims 1-6, 10 and 16 have been rejected under 35 U.S.C. §102(b) as being anticipated by Varma et al. It is specifically alleged that Varma et al. disclose the production of cells of the microorganism *E. coli* in the presence of the alternative oxidant source acetate, under aerobic conditions such that the strain utilizes the alternative oxidant source.

Applicant respectfully disagrees with the rejection of claims 1-6, 10 and 16. The Varma et al. reference describes a predictive algorithm to apply the flux balance model to describe unsteady-state growth and by-product secretion in aerobic batch, fed-batch and anaerobic batch cultures. The experiment involved the use of *E. coli* W3110. The culture media used includes Na_2HPO_4, KH_2PO_4, NaCl, NH_4Cl, $MgSO_4$, $CaCl_2$, $FeCl_3$. Glucose was used as the carbon source. The Varma et al. reference discloses that *E. coli* W3110 secretes acetate and, under certain circumstances, reutilizes the secreted acetate as a ***carbon source***.

In contrast, the present invention provides a process for producing biological products from cells by providing cells that can utilize both oxygen and an alternative oxidant for cellular respiration. The cells are inoculated into a culture media and maintained at a desired pH and temperature. The culture media is aerated with oxygen and supplied with an alternative oxidant source. The oxygen is supplied at a rate, such that when the oxygen requirements for cellular respiration of the microorganisms within the culture medium is less than the maximum rate of oxygen supply to the culture medium, then the microorganisms will substantially utilize oxygen for cellular respiration, and when the oxygen requirements for cellular respiration of the microorganisms within the culture medium is greater than the maximum rate of oxygen supply to the culture medium, then a portion of the microorganisms within the culture medium will utilize the alternative oxidant source for cellular respiration.

Suitable carbon sources for cellular respiration include, for example, glucose and acetate. Suitable alternative oxidant sources include nitrates, nitrites, sulfates, sulfites, carbonates, bicarbonates, fumarates, sulfur, manganic ion, ferric ion, selenate, dimethyl sulfoxide, arsenate, trimethylamine-N-oxide and glycine.

The Varma et al. reference does not teach to supply the culture with oxygen and an alternative oxidant source. Varma et al. also do not teach to supply oxygen at a rate, such that when the oxygen requirements for cellular respiration of the microorganisms within the culture medium is less than the maximum rate of oxygen supply to the culture medium, then the microorganisms will substantially utilize oxygen for cellular respiration, and when the oxygen requirements for cellular respiration of the microorganisms within the culture medium is greater than the maximum rate of oxygen supply to the culture medium, then a portion of the microorganisms within the culture medium will utilize the alternative oxidant

source for cellular respiration. It has been alleged that Varma et al. teach that acetate is utilized as the alternative oxidant source. Applicant respectfully submits that this is not correct. Acetate is clearly utilized as an alternative ***carbon source*** for the *E. coli*. Varma et al. teach "It is generally assumed that the presence of glucose represses the utilization of other substrates. In contrast, we observed in both experiments and in the model that a sufficiently high cell density can result in the simultaneous consumption of glucose and acetate." See p. 3730, column 2, paragraph 3. Clearly, acetate is an alternative ***carbon source*** of the *E. coli*, when glucose was depleted or under other conditions that permitted simultaneous utilization of glucose and acetate. Accordingly, claims 1-6, 10 and 16 are not anticipated, as the Varma et al. reference does not teach each and every limitation of the claimed invention. Applicant, therefore, respectfully requests that the rejection of claims 1-6, 10 and 16 be withdrawn.

Claims 1-10, 13, 15, 17-20, 22, 27-29 and 31-34 have been rejected under 35 U.S.C. §102(b) as being anticipated by United States Patent No. 5,501,996 to Giani et al. It is specifically alleged that Giani et al. teach production of cells of *P. aeruginosa* in the presence of the alternative oxidant source $NaNO_3$ under aerobic conditions.

Applicant respectfully disagrees with this rejection. United States Patent No. 5,501,996 teaches process for production of rhamnolipids and recovery of L-Rhamnose from the produced rhamnolipids. The rhamnolipids are produced by *P. aeruginosa* under aerobic conditions, wherein oxygen is continually provided to the cells for cellular respiration. It is widely known that cells must be provided with a liquid culture medium containing required nutrients to support cellular growth. Giani et al. recognized this and teaches "[A]part from vegetable oil, the media must additionally contain one or more nitrogen sources, sulfate and magnesia ions, and potassium and chloride ions, one or more phosphorous sources and trace elements." See Column 5, lines 25-34. While Giani et al. teach that $NaNO_3$ can be included in the culture medium, the $NaNO_3$ is not included as an alternative oxidant source for cellular respiration, but it is most likely added as a nitrogen source for protein synthesis. In fact, there is no teaching in Giani for the *P. aeruginosa* to utilize an alternative oxidant source in the absence of oxygen, as oxygen is continuously provided to the culture medium. The Giani et al reference does not teach to supply the culture with oxygen and an alternative oxidant source. Giani et al. also do not teach to supply oxygen at a rate, such that when the oxygen

requirements for cellular respiration of the microorganisms within the culture medium is less than the maximum rate of oxygen supply to the culture medium; then the microorganisms will substantially utilize oxygen for cellular respiration, and when the oxygen requirements for cellular respiration of the microorganisms within the culture medium is greater than the maximum rate of oxygen supply to the culture medium, then a portion of the microorganisms within the culture medium will utilize the alternative oxidant source for cellular respiration. Applicant, therefore, respectfully submits that the claims are not anticipated by the Giani et al. reference.

35 U.S.C. §103(a) Rejection

Claims 1-34 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Giani et al, in view of Brock and Wagner et al. It is specifically alleged that (1) Giani teaches production of *P. aeruginosa* under aerobic conditions in the presence of $NaNO_3$, (2) Brock teaches oxidants of fumarate, sulfate, ferric ion and nitrite, and (3) Wagner et al. teach that it is known in the art to provide nutrient media with acids, including malonate, succinate, pyruvate or malate, or fatty acids, such as stearic acid, and (4) Wagner et al. teaches nutrient limitation of bacteria culture media. It is, therefore, alleged that it would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the process of Giani et al, by the substitution of nitrate by other oxidants, as suggested by Brock, as well as the use of nutrient limitation on various carbon sources, as suggested by Wagner et al.

Applicant respectfully disagrees with the rejection of claims 1-34. The teachings of Giani et al. are discussed above with respect to the rejection under 35 U.S.C. 102(b). This discussion is not reproduced here, but all of the discussed distinctions between Giani et al and the present invention apply equally to the rejection under 35 U.S.C. 103(a). Brock teaches various alternative electron acceptor for anaerobic respiration. Wagner et al. teach a method to produce rhamnolipids under aerobic conditions. Brock, however, does not teach, suggest or provide motivation for a combined process that utilizes both aerobic respiration and anaerobic respiration. Giani et al. does not teach that $NaNO_3$ is an alternative oxidant source for anaerobic respiration. Wagner et al. teaches to produce rhamnolipids for *P. aeruginosa* under aerobic conditions. Nowhere in the Wagner et al. reference does it disclose, teach,

suggest or provide motivation to include an alternative oxidant source in the culture medium. This is because Wagner et al. is strictly limited to aerobic processes. Accordingly, there would be no need for an alternative oxidant source in a wholly aerobic process. Therefore, there is no motivation to combine Wagner et al. with Giani et al. Even if Wagner et al. and Giani et al. were combined, the present invention is still not disclosed by this combination. Both references teach wholly aerobic processes, and both references do not teach the addition of an alternative oxidant source for use by microorganisms during anaerobic cellular respiration. Applicant, therefore, respectfully requests that the rejection of claims 1-34 be withdrawn.

Should the Examiner have any questions, the undersigned attorney would welcome a telephone call.

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1-16-03
Date

APPENDIX

MARKED UP VERSION SHOWING AMENDMENTS TO APPLICATION

IN THE SPECIFICATION

The paragraph at page 11, lines 24-28 spanning page 12, lines 1-6 has been amended as follows:

Facultative aerobic bacteria are those species of bacteria that can either utilize oxygen for respiration purposes under aerobic conditions, or can utilize alternative oxidants other than oxygen for respiration purposes in the absence of oxygen. Suitable species of facultative aerobic bacteria that may be used in the present invention include, but are not limited to, nitrate/nitrite respiration bacteria such as *Pseudomonas aeruginosa, Pseudomonas fluorescens, Paracoccus denitrificans, Micrococcus halodenitrificans, Klebsiella aerogenes, Escherichia coli*, and the like; hyperthermophilic Archaea bacteria such as *Acidianus*; and the fumarate respiration bacteria such as *Wolinella succinogenes,* [*Desulfovibrio gigas, Clostridia,*] *Escherichia coli* and *Proteus rettgeri*. A preferred facultative aerobic bacterium is that of the genus *Pseudomonas*.

IN THE CLAIMS

Claim 6 has been amended as follows:

6. (amended) The process of claim [5] 4, wherein the [facultative aerobe] bacteria is selected from a genus selected from the group consisting of *Pseudomonas, Paracoccus, Micrococcus, Klebsiella, Escherichia, Acidianus, Campylobacter, Wolinella,* [*Desulfovibrio, Clostridium,*] and *Proteus*.